1: Biotechniques 1997 May;22(5):938-46

An iterative and regenerative method for DNA sequencing.

Jones DH.

University of Iowa, Iowa City, USA. douglas-h-jones@uiowa.edu

This paper presents, to our knowledge, the first iterative DNA sequencing method that regenerates the product of interest during each iterative cycle, allowing it to overcome the critical obstacles that impede alternative iterative approaches to DNA sequencing: loss of product and the accumulation of background signal due to incomplete reactions. It can sequence numerous double-stranded (ds) DNA segments in parallel without gel resolution of DNA fragments and can sequence DNA that is almost entirely double-stranded, preventing the secondary structures that impede sequencing by hybridization. This method uses ligation of an adaptor containing the recognition domain for a class-IIS restriction endonuclease and digestion with a class-IIS restriction endonuclease that recognizes the adaptor's recognition domain. This generates a set of DNA templates that are each composed of a short overhang positioned at a fixed interval with respect to one end of the original dsDNA fragment. Adaptor ligation also appends a unique sequence during each iterative cycle, so that the polymerase chain reaction can be used to regenerate the desired template-precursor before class-IIS restriction endonuclease digestion. Following class-IIS restriction endonuclease digestion, sequencing of a nucleotide in each overhang occurs by template-directed ligation during adaptor ligation or through a separate template-directed polymerization step with labeled ddNTPs. DNA sequencing occurs in strides determined by the number of nucleotides separating the recognition and cleavage domains for the class-IIS restriction endonuclease encoded in the ligated adaptor, maximizing the span of DNA sequenced for a given number of iterative cycles. This method allows the concurrent sequencing of numerous dsDNA segments in a microplate format, and in the future it can be adapted to biochip format.

PMID: 9149879 [PubMed - indexed for MEDLINE]